

Distribution of P metabolites and compartmentation in soybean nodules as studied by electron microscopy and ^{31}P NMR spectroscopy

D. ROLIN, P. PFEFFER, J. SCHIMDT, R. BOSWELL, P. COOKE and S. JONES
 Eastern Regional Research Center, USDA, ARS Philadelphia, PA 19118, USA

Introduction

In a previous study (Rolin *et al.*, 1989) we established the optimum parameters for obtaining ^{31}P spectra of functioning soybean nodules. The purpose of the present study was to investigate nodule compartmentation using *in vivo* ^{31}P NMR in conjunction with electron microscopy to establish the distribution of the metabolites in nodules.

Results and discussion

The root nodules consist of a heterogeneous cell population. The cortex surrounding the central matrix represents 50% of the total volume of the nodule. The active, N_2 fixing tissue is a central core of enlarged host parenchyma cells, which are packed with the endosymbiotic bacteria. The cells of the central tissue form a compact mass with the uninfected and infected cells. The infected host cells of the nodule central tissue are densely packed within the peribacteroid membrane.

During *in vivo* ^{31}P NMR experiments, all of the examined tissues remained alive for at least 20h when the perfusion medium contained 50mM glucose and was saturated with O_2 . Eighty-seven percent of the total mobile Pi in the 9 week-old nodules was in the acidic compartment whose Pi shift corresponds to pH 5.5. A ^{31}P spectrum of the carefully excised cortex layer showed that 59% of the total mobile Pi of the nodules resided in the vacuole of the cortical cells. The spectrum of 7 week-old intact nodules shows that 77% of the total mobile Pi is present in the acidic compartment. The central matrix of

the nodule was separated from the cortical cells by careful excision with a scalpel. Only 34% of the total mobile Pi in the matrix was found to be present in the vacuole of the uninfected cells, the only acidic compartment in the central matrix. In addition, it may be possible to estimate the relative quantity of mobile Pi in the bacteroid. In the *in vivo* spectrum of isolated bacteroids, the resonance at 2.30 ppm represents the cytoplasmic Pi of the bacteroids corresponding to a pH of 6.85. The bacteroids have a relatively more acidic cytoplasm than the observed pH 7.4 of the host plant cells. However, these pH dependent shift differences are not resolved in the nodule spectra due to broader peak widths. The relative distribution of cytoplasmic and vacuolar Pi in nodules was compared in 4 to 12 week-old nodules. In the earlier growth stages (4 week old), most of the Pi was found in the cytoplasm (61%). After 7 weeks, the majority of the Pi (77%) was stored in the vacuole of the host cells (cortical plus uninfected cells). Since most of the Pi of nodules at 4 weeks was present in the metabolically active cytoplasm, absorption of Pi probably did not greatly exceed the cell's needs. Throughout the growth period from 4 to 12 weeks the vacuolar Pi of the uninfected cells remained relatively constant at 32% of the total Pi. However, Pi was preferentially accumulated in the vacuole of the cortical cells (61% of the total Pi for the 12 week-old nodules) at the expense of the cytoplasmic Pi.

Reference

Rolin D *et al.* 1989 Plant Physiol. 89, 1238–1246.

Effect of acidified soil on symbiosis in soybeans

G.R. CLINE and KARAN KAUL

Community Research Service, Kentucky State University, Frankfort, KY 40601, USA

To improve the tolerance of soybean plants to acidic soils, it is important to determine which acidic soil infertility factors are most detrimental and whether the plant or the symbiotic relationships with *Bradyrhizobium japonicum* is more adversely affected. The objectives of this study were (1) to compare the effects of acidity and Al toxicity on soybeans grown in soil and (2) to determine if the plant or the symbiosis is more susceptible to acidic soil conditions.

A completely randomized 3 soil acidity \times 5 N treatment factorial experimental design was used with each treatment combination replicated in three pots containing three plants each. The three soil acidity treatments consisted of nonacidified Maury silt loam soil and soil acidified with $7.5 \text{ g kg}^{-1} \text{ Al}_2(\text{SO}_4)_3$ or $1.7 \text{ g kg}^{-1} \text{ S}_2$. The N treatments included a nonamended control, addition of 100 mg N kg^{-1} soil as NH_4NO_3 , and inoculation with commercial *B. japonicum* or the locally isolated KY1 strain plus the addition of 0.05 mg kg^{-1} Mo to the soil as NaMoO_3 .

Treatment of soil with $\text{Al}_2(\text{SO}_4)_3$ resulted in a soil pH of 4.8, $30 \mu\text{M}$ soluble Al, and $0.77 \text{ cmol}_c \text{ kg}^{-1}$ KCl-Al. Soil amended with S_2 was more acidic, pH 4.6, but only contained about half as much Al, $14 \mu\text{M}$ soluble Al and $0.37 \text{ cmol}_c \text{ KCl-Al}$.

Soil acidification with either acidulent did not

significantly reduce shoot or root dry weights of plants receiving inorganic N, but acidification significantly ($\alpha = 0.05$) reduced shoot and root dry weights, nodule dry weights and number, and foliar N content of inoculated plants. Thus, under acidic soil conditions the adversely-affected symbiosis appeared to be the primary cause of reduced plant growth. Inoculated plants from S_2 -amended soil were significantly ($\alpha = 0.05$) smaller and less well nodulated than those grown in $\text{Al}_2(\text{SO}_4)_3$ -amended soil which was less acidic but contained more soluble Al than the former soil. Thus, acidity appeared to be more detrimental to plant growth than Al toxicity. Addition of Mo to inoculated plants was not beneficial.

Addition of the two soil acidulents also increased electrical conductivity (2.6 mS cm^{-1}), Ca (1.6 mM), and SO_4 -ions (1.9 mM). However, these conditions did not appear to adversely affect the plant or bacterium since dry weights of N-fertilized plants were not significantly reduced by soil acidification and growth of KY1 *B. japonicum* in pure culture was not affected when subjected to similar conditions.

It was concluded that (1) acidity appeared to affect soybeans more so than did Al toxicity and (2) the induced acidified soil conditions affected the symbiosis more than the host plant.